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Variation in Pollination: Causes and Consequences for Plant Reproduction

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ABSTRACT: Pollen dispersal by animals varies extensively because of differences in pollinator visitation rates among plants, dissimilar pollination by the various pollinators that visit individual plants, and stochastic variation in deposition as an individual pollinator disperses a plant's pollen to subsequently visited recipient flowers. Such variation reduces expected female and male success if seed production decelerates with increasing pollen receipt, because less than average receipt diminishes mean seed production more than copious pollination increases it (Jensen's inequality). We report empirical studies of the nature and magnitude of pollen dispersal variance, which provide the basis for a numerical model of the consequences of dispersal for expected seed production. Model fitting revealed that dispersal of *Brassica napus* pollen by bumblebees and especially butterflies exhibited much more variation than is expected of a binomial process and was best modeled as a beta-binomial process with a constant mean. Overdispersion arose primarily during pollen dispersal by individual insects, since differences between individuals of the same pollinator type were limited. Our model revealed variance limitation as a previously unrecognized, substantial, and ubiquitous component of pollen limitation of seed production. Variance limitation should select for floral traits that increase pollinator visitation, reduce dispersal variance, or reduce the postpollination nonlinearities that cause Jensen's inequality.

Keywords: Jensen's inequality, model selection, pollination, pollen limitation.

Introduction

Pollination is highly uncertain. For animal-pollinated species with granular pollen, typically <1% of the pollen that pollinators remove from flowers reaches conspecific stigmas (Harder and Johnson 2008). In addition, pollen export and import vary extensively among plants and among flowers within plants (Waser and Price 1984; Thomson 1986). For example, Herrera (2002) estimated that roughly

half of the variance in the number of pollen tubes in *Helleborus foetidus* pistils sampled from 29 populations in three regions of the Iberian Peninsula arose from differences among flowers on individual plants. In general, variation in pollen dispersal by animals arises from three sources: differences in pollinator visitation, differences among pollinators, and stochastic variation during dispersal by individual pollinators. Visitation differences arise from both intrinsic differences in a plant's or flower's attractiveness, such as flower and display size, and variation in the local density of conspecifics and other plant species that compete for pollinators or facilitate their attraction (e.g., Ohashi and Yahara 2001; Bell et al. 2005; Grindeland et al. 2005; Ghazoul 2006; Hegland and Boeke 2006; Ishii and Harder 2006). Relevant differences among pollinators of the same or different species include average pollen removal and deposition per visit, the average incidence and intensity of processes that cause pollen loss during transport (e.g., grooming), and the frequency and distance of movement within and among plants (e.g., Wilson and Thomson 1991; Stone 1996; Castellanos et al. 2003; Fumero-Cabán and Meléndez-Ackerman 2007). Finally, stochastic variation in pollen removal, loss, and deposition as individual pollinators carry pollen from one flower or plant to others can arise from differing pollinator position and visit duration and from variation in stigma and anther positions (Lertzman and Gass 1983; Waser and Price 1984; Thomson 1986; Morris et al. 1994; Cresswell 1999). Curiously, although the causes of variation in pollen dispersal are widely recognized, its ecological and evolutionary consequences have received little attention (although see Lertzman and Gass 1983; Galen and Rotenberry 1988; Burd 1995; Harder and Wilson 1998).

Variation in pollen dispersal can affect the expected female and male success of plants if seed production and siring success vary nonlinearly with pollen deposition on stigmas (e.g., Wilson and Harder 2003). This association arises because of Jensen's inequality (Jensen 1906; also see Smallwood 1996; Ruel and Ayres 1999; Pásztor et al. 2000), according to which the mean outcome of a nonlinear func-

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tion with a variable input does not equal the value of the function for the mean input. For example, consider figure 1, which depicts the relation of the number of pollen tubes at the base of *Chamerion angustifolium* styles to the number of pollen grains deposited on their stigmas (A. S. Nixon and L. D. Harder, unpublished data). Because this relation decelerates strongly, the average number of pollen tubes for two flowers that differ in pollen receipt by 700 grains above and below the mean (*midpoint between the two dashed horizontal lines*) is 8% smaller than the number of pollen tubes expected for the average pollen receipt (*solid horizontal line*). More generally, the average number of pollen tubes in all pistils is 13.2% smaller than expected for the average pollen receipt. According to Jensen's inequality, reduction in the average output of a nonlinear process owing to variation in input arises whenever the output is a decelerating function of the input. Such relations probably commonly affect average reproductive performance in plant populations because of nonlinear (decelerating) processes during pollen tube development in the style (fig. 1), competition among pollen tubes to fertilize limited ovules, competition among developing seeds for maternal resources, and competition among seeds for establishment sites (e.g., Waser and Price 1991; Mitchell 1997; Aizen and Basilio 1998; Aizen and Harder 2007; Harder et al. 2008). Together, such nonlinearities will accentuate the effect of variation in pollen dispersal on expected female and male performance. For example, Wilson and Harder (2003) demonstrated theoretically that separation of the sexes increases the spatial variance in pollen dispersal compared with that experienced by hermaphrodites, so Jensen's inequality greatly restricts the conditions under which a dioecious species can outcompete a hermaphroditic species with otherwise similar reproductive characteristics. Such effects bear consequences for both the dynamics of plant populations and selection on floral traits and sexual systems.

Dispersal variance arises as the aggregate outcome of pollen transport by individual pollinators. During dispersal of granular pollen, some pollen removed by a pollinator from a specific donor flower remains on the pollinator as it subsequently visits a series of recipient flowers. Depletion of donor pollen on the pollinator by loss during transport and deposition on recipient stigmas produces a generally declining trend in dispersal of donor pollen to successive recipients (Harder and Barrett 1996). Bateman (1947) first modeled this trend as a geometric decay process:

$$E[d_v] = Dp(1 - p)^{v-1}, \quad (1)$$

where D is the total number of donor pollen grains eventually deposited on recipient stigmas by a single pollinator, $E[d_v]$ is the expected number of donor grains deposited

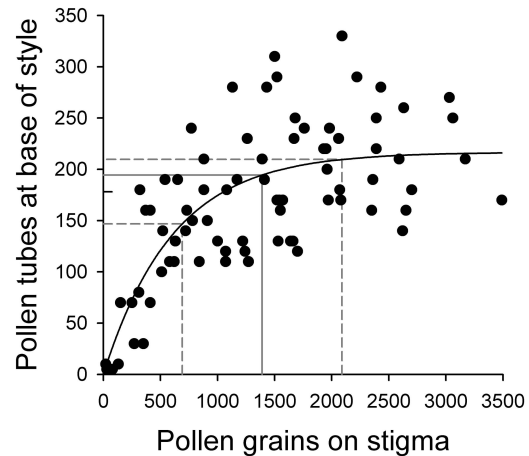


Figure 1: Effect of variation in pollen receipt (X-axis) on the number of pollen tubes at the base of the style (Y-axis) for *Chamerion angustifolium* and the consequences for Jensen's inequality. The solid regression line represents the maximum likelihood fit of $\hat{y} = 216.6(1 - e^{-0.00164x})$, assuming a negative binomial error distribution. The straight solid line maps the number of pollen tubes expected given average pollen receipt, whereas the straight dashed lines map expected pollen tube numbers of flowers that received 700 fewer or more pollen grains than the mean. The tick inside the Y-axis indicates the average of the latter two expectations, which is lower than the expected number of pollen tubes, assuming average pollen receipt (Jensen's inequality).

on the stigma of recipient flower v in the visit sequence, and p is the expected proportion of pollen remaining on the pollinator that is deposited during each visit (deposition fraction; also the ratio of grains deposited on a stigma relative to those deposited on the stigma of the previous recipient). Consequently, $1 - p$ represents the average proportion of ultimately deposited pollen that is carried over on the pollinator between flower visits (carryover fraction; Morris et al. 1994). Subsequent analysis of pollen dispersal by bumblebees and hummingbirds suggested that average deposition commonly declined faster than expected for geometric decay among the initial recipient flowers in a sequence but then persisted for more recipients than expected (Morris et al. 1994; Harder and Barrett 1996). For analyses that consider the average number of pollen grains deposited on the first, second, and subsequent recipients visited by a sample of pollinators (e.g., Harder and Barrett 1996), such apparently "long-tailed" dispersal could arise simply as an artifact of averaging geometric decay processes governed by different parameters for each pollinator (Harder and Wilson 1998; appendix). Given this potential impact of between-pollinator variation, analysis of pollen dispersal must consider individual rather than averaged sequences. Nevertheless, dispersal by individual pollinators could be more complicated than simple geometric decay because of pol-

linator contact with stigmas of some flowers but not others (Lertzman and Gass 1983; Morris et al. 1995), pollinator grooming during flights between flowers (Harder and Wilson 1998), and the accumulation of pollen in layers on pollinators' bodies (Morris et al. 1995; Harder and Wilson 1998).

In this article, we consider three sources of variation in pollen dispersal and their general consequences for plant mating: differences among pollinator types, differences among individual pollinators of the same type, and stochastic pollen deposition on flowers visited by individual pollinators. This study is based on observations of pollen dispersal between donor and recipient plants of *Brassica napus* L. cv. Westar by worker bumblebees (*Bombus melanopygus* Nylander) and male butterflies (*Pieris rapae* [L.]). We first derive a series of pollen dispersal models that extend Bateman's model by explicitly depicting each of the three sources of variation. We then fit these models to our data and use model selection to identify which sources of variation contribute most to patterns of pollen dispersal. Pollen loss during transport could not be measured, so after presenting the empirical results, we present simulation results that assess the effects of loss on model fitting. On the basis of the empirical results, we then examine the implications of variation among and within pollinators on plant mating with a numerical model. Together, our empirical and theoretical results provide guidance on the analysis of pollen dispersal and, more importantly, expose new perspectives on the extent and causes of dispersal variance, its consequences for average reproductive output in plant populations, and its potential roles in the evolution of floral characteristics.

Material and Methods

Plants and Pollinators

To study dispersal of *Brassica napus* pollen, we used individual transgenic plants as pollen donors and wild-type plants as pollen recipients. Transgenic plants were selfed progeny from a T1 transgenic line (Sta-44) containing a genetic construct of the β -glucuronidase (GUS) reporter gene (Jefferson et al. 1987) linked to a pollen-specific promoter element (Hong et al. 1997). The construct, which is expressed in mature male gametophytes (pollen grains and pollen tubes), codes for β -glucuronidase, which cleaves β -D-glucuronide substrate, producing a blue precipitate in the area of gene expression—in our case, within the pollen grain. All transgenic plants used in our experiments contained multiple insertions of the GUS gene and were screened to select only plants with strong and consistent blue color in all assayed pollen grains. Other than the expression of blue color in pollen grains treated with

substrate-containing reagent, the transgenic plants used in our experiments could not be distinguished from wild-type plants.

The bumblebees used in our experiments were from a single colony initiated by a wild queen in a domicile (Hobbs et al. 1960). When the colony had about 30 workers, we moved it to the laboratory and marked each worker by gluing a numbered plastic tag to its thorax. The bees' nest box was placed inside a $2 \times 2.75 \times 2.25$ -m flight cage, where workers had access to wild-type *B. napus* plants between experimental trials. We also provided the colony with supplemental pollen and 50% sucrose solution.

The butterflies were from a captive colony maintained as described by Webb and Shelton (1988). Butterflies were marked uniquely on their forewings with a felt-tipped pen. Individuals used in experimental trials were first exposed to wild-type *B. napus* plants in a $2 \times 1.5 \times 1.5$ -m flight cage. We used only male butterflies to eliminate interruption of experimental trials due to oviposition.

Experimental Protocol

We quantified pollen removal and dispersal in an indoor flight cage, illuminated brightly by 40-W, full-spectrum fluorescent tubes, using zigzag arrays of potted *B. napus* plants, with one transgenic donor and a series of up to 10 wild-type recipient plants. We ensured that the plants used during our experiment had similar characteristics, except for some experimental differences between trials with bees. Before a trial, we trimmed plants so that they presented one inflorescence with about eight flowers with fully dehiscid anthers. Before each butterfly trial, we both deprived butterflies of nectar for at least 2 h and depleted nectar from potential recipient flowers by allowing 10 butterflies to visit the wild-type plants, because butterflies seldom visited more than one flower with accumulated nectar per hour. After this preexposure, flowers retained most of their pollen. To avoid disturbing pollen on flowers of the donor plant, we extracted their nectar with filter paper wicks. Unlike butterflies, bumblebees would not visit depleted flowers consistently, so we used previously unvisited donor and recipient plants for their trials. To examine the effects of pollen transport loss caused when bees groom their bodies after removing abundant pollen from flowers (Harder 1990) on pollen dispersal characteristics, we brushed anthers on all recipient plants for 13 of the 46 trials involving bumblebees (see Thomson 1986).

During each trial, an individual pollinator visited three types of plants in sequence. First, the pollinator had access to only wild-type plants to allow it to accumulate pollen on its body. These plants were removed once the pollinator moved to the single, transgenic donor plant. Similarly, while the pollinator subsequently visited flowers on recip-

ient plants, we removed the donor plant. To limit the pollinator from revisiting flowers, we replaced visited plants with unvisited plants while the insect was on a distant plant in the array. Trials usually ended when the pollinator ceased foraging (bee returned to nest, butterfly alighted for a protracted period). We videotaped each trial to record the visit sequence. Each butterfly was used for a single trial, whereas five bees were used twice among the 21 dispersal trials reported here.

Pollen dispersal was quantified by counting stained donor pollen grains on the stigmas of each visited flower. We excised a flower's stigma with the distal 2 mm of style and mounted it upright on water agar in the well of a tissue culture plate. After covering the plate, we left the stigmas at room temperature for 2 h to allow pollen to germinate and pollen tubes to anchor grains to the stigma. We then added 0.5 mL of fresh reagent containing the β -D-glucuronide substrate to each well, covering the stigma completely, and incubated the stigmas at 37°C for 50 min, by which time blue color had developed fully in transgenic grains. We counted the transgenic and wild-type pollen grains on each mounted stigma using a dissecting microscope. We also digitally photographed stigmas that had received many donor pollen grains so that they could be recounted later.

Models of Pollen Dispersal

Our theoretical and statistical analyses consider the following model, or variants of it (see table 1), that follow the R pollen grains carried away from a specific donor flower by a single pollinator as it subsequently visits a sequence of recipient flowers. Suppose that while moving between flowers, both donor and nondonor pollen grains become unavailable for transfer to stigmas with probability u because they actively or passively fall off the pollinator or are moved to sites on the pollinator that do not contact stigmas. Also, suppose that while visiting a flower, each remaining pollen grain on the pollinator is deposited on the stigma with probability t . If the probabilities of loss

and deposition are constant among flower visits, then the pollinator will carry an average of $R_v = R(1 - u)^v(1 - t)^{v-1}$ donor pollen grains immediately before visiting the v th recipient beyond the donor. If pollen grains move independently of each other, then both loss and deposition are binomial processes. In this case, when exactly R_v donor pollen grains remain on the pollinator before it visits recipient v , the probability distribution of the number of these pollen grains deposited on the stigma of recipient v , denoted d_v , is

$$\Pr(d_v | R_v, t) = \text{bin}(d_v | R_v, t) \\ = \frac{R_v!}{d_v!(R_v - d_v)!} t^{d_v} (1 - t)^{R_v - d_v}, \quad (2a)$$

which has a mean and variance of

$$E[d_v] = R_v t, \quad (2b)$$

$$\text{Var}[d_v] = R_v t(1 - t) \quad (2c)$$

(see fig. 2A, binomial distribution). Equation (2b) is a variant of Bateman's (1947) geometric decay model (eq. [1]) and the two models are equivalent by setting $p = t + u(1 - t)$. Equation (2c) describes the variation in donor grains deposited v visits after the donor flower if the number of grains on the pollinator immediately before deposition was known; however, stochastic loss before visit v will result in uncertainty in R_v . When loss is uncertain, the variation in deposition during visit v , given that the pollinator initially carried away R donor grains, is

$$\text{Var}[d_v] = R_v t[1 - t(1 - u)^v(1 - t)^{v-1}]. \quad (2d)$$

In contrast to the binomial model, pollen grains may not act independently, since they are often sticky and form clumps (Harder and Johnson 2008), and the deposition probability may be heterogeneous between flower visits because pollen-bearing sites on a pollinator's body differ in their susceptibility to grooming or chance of stigma

Table 1: Description of the eight models fit to the pollen dispersal data

Model	Free parameters	Model description
Binomial:		
B(−)	\bar{p}_1	Constant probability of deposition
B(c)	c, \bar{p}_1	Constant deposition plus possible noncontact
B(v)	$\bar{p}_1, \bar{p}_\infty, \lambda$	Visit-dependent probability of deposition
B(v + c)	$c, \bar{p}_1, \bar{p}_\infty, \lambda$	Noncontact and visit-dependent deposition
Beta-binomial:		
BB(−)	\bar{p}_1, ϕ	Variable, visit-independent probability of deposition
BB(c)	c, \bar{p}_1, ϕ	Variable deposition plus possible noncontact
BB(v)	$\bar{p}_1, \bar{p}_\infty, \lambda, \phi$	Variable and visit-dependent probability of deposition
BB(v + c)	$c, \bar{p}_1, \bar{p}_\infty, \lambda, \phi$	Noncontact and variable, visit-dependent deposition

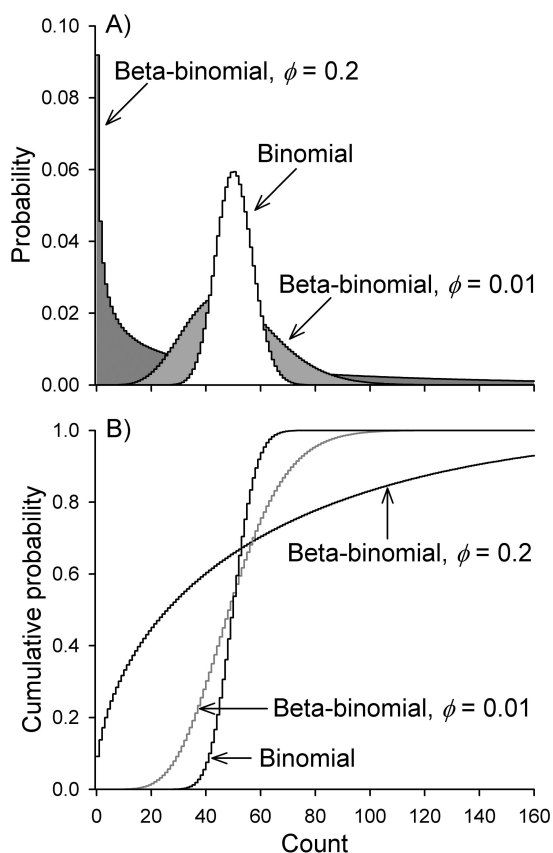


Figure 2: Binomial (white) and beta-binomial (gray) probability distributions (A) and cumulative probability distributions (B) for pollen deposition on stigmas given 500 pollen grains on the pollinator immediately before a visit and a mean probability of deposition per pollen grain of $\bar{p} = 0.1$.

contact (Kimsey 1984), flowers differ in features that determine stigma-pollinator contact (Waser and Price 1984; Murcia 1990), and the pollinator's positioning varies between visits (Lertzman and Gass 1983; Fetscher et al. 2002). Pollen dispersal characteristics may also vary among pollinator species (Castellanos et al. 2003) and among individuals of the same species, depending on their size, activity level, foraging experience, and so on (Murcia 1990). Such processes could be modeled explicitly (e.g., Morris et al. 1995; Harder and Wilson 1998); however, fitting such models to data requires measurements of the underlying processes, which is usually difficult or impossible. A more practical approach recognizes that these biological processes generate specific probability density distributions for the probabilities that govern the binomial processes of pollen loss and deposition. The beta distribution is well suited to this purpose. For example, according to this distribution, the probability of pollen grains being transferred

from a pollinator to a stigma during visit v , t_v , is distributed as

$$\text{beta}(t_v | \bar{t}_v, \phi_t) = \frac{\Gamma(a+b)}{\Gamma(a)\Gamma(b)} t_v^{a-1} (1-t_v)^{b-1}, \quad (3)$$

where \bar{t}_v is the average transfer probability, $\Gamma(x)$ is the complete gamma function (see appendix), $a = \bar{t}_v/\phi_t$, $b = (1 - \bar{t}_v)/\phi_t$, and ϕ_t is a positive parameter that characterizes the variance of t_v , such that $\text{Var}[t_v] = \bar{t}_v(1 - \bar{t}_v)\phi_t/(1 + \phi_t)$. In the limit as ϕ_t approaches 0, t_v is constant and equal to \bar{t}_v . Now, if the pollinator carries R_v donor pollen grains immediately before the v th flower visit, the probability of d_v grains being deposited on the flower's stigma is given by the beta-binomial distribution:

$$\begin{aligned} \Pr(d_v | R_v, \bar{t}_v, \phi_t) &= \text{betabin}(d_v | R_v, \bar{t}_v, \phi_t) \\ &= \int_{t_v=0}^1 \text{beta}(t_v | \bar{t}_v, \phi_t) \text{bin}(d_v | R_v, t_v) dt_v \\ &= \frac{\Gamma(R_v + 1)\Gamma(a+b)\Gamma(d_v + a)\Gamma(R_v - d_v + b)}{\Gamma(d_v + 1)\Gamma(R_v - d_v + 1)\Gamma(a)\Gamma(b)\Gamma(R_v + a + b)} \end{aligned} \quad (4)$$

(see fig. 2A, beta-binomial distributions), which has mean $R_v \bar{t}_v$, as for the binomial case, and variance $\text{VIF} \times \text{Var}[d_v]$, where $\text{Var}[d_v]$ is the variance of the binomial distribution with the same mean (eq. [2c]) and $\text{VIF} = 1 + (R_v - 1)\phi_t/(1 + \phi_t)$ is the variance inflation factor describing the increase in variance relative to the binomial distribution (Richards 2008). Figure 2A clarifies that as ϕ_t increases, deposition of zero pollen grains on a given recipient stigma becomes increasingly probable, even if the pollinator carries many pollen grains. Pollen loss between flower visits can be modeled similarly.

Observations of pollen dispersal by individual pollinators often include stigmas from visited flowers that receive no donor pollen, even though stigmas of subsequently visited flowers receive many. Such null visits could arise either because the pollinator does not contact the stigma while visiting a flower or because contact occurred but did not result in pollen transfer. To account for these two possibilities, we also considered zero-inflated models (Martin et al. 2005) that included a probability c of no stigma contact, so that the probability of d_v pollen grains being deposited on the stigma of the v th flower visited after the donor is

$$\begin{aligned} \Pr(d_v | R_v, c, \bar{t}_v, \phi_t) &= \\ \begin{cases} c + (1-c)\text{betabin}(d_v | R_v, \bar{t}_v, \phi_t) & \text{if } d_v = 0 \\ (1-c)\text{betabin}(d_v | R_v, \bar{t}_v, \phi_t) & \text{if } 1 \leq d_v \leq R_v \end{cases} \end{aligned} \quad (5)$$

In theory, one could estimate the functional forms for \bar{t}_v and \bar{u}_v and the constants c , ϕ_p , and ϕ_u if the numbers of grains immediately before and after flower probing were known, but in practice, such detailed information regarding pollen loads will be unknown (and unknowable). Instead, when donor pollen can be distinguished from non-donor pollen, such as by the GUS-reporter gene technique used in our experiment, observations include the number of donor grains on stigmas, d_v , for sequences of visits to recipient flowers by individual pollinators. Summing all d_v gives the total number of donor grains dispersed to stigmas, D . Hence, the total number of donor grains on the pollinator that will ultimately be deposited on stigmas after it visits the v th recipient is

$$D_v = D - \sum_{i=1}^{v-1} d_i. \quad (6)$$

Given the sequence of deposition data $\mathbf{d} = \{d_1, \dots, d_v\}$, a single probabilistic model analogous to the pollen transfer model can be fit using maximum likelihood. Specifically, we consider the model described by equation (5), with \bar{t}_v in equation (4) replaced by \bar{p}_v and ϕ_t replaced by ϕ . As described below, we will consider the effects of not accounting explicitly for pollen loss by simulation. The possibility that the mean probability of donor pollen being deposited on a stigma changes consistently with the position of the recipient in the visitation sequence, v , is modeled by

$$\bar{p}_v = \bar{p}_\infty - (\bar{p}_\infty - \bar{p}_1) \exp[-\lambda(v-1)], \quad (7)$$

where \bar{p}_1 , \bar{p}_∞ , and λ are nonnegative constants. Equation (7) proposes that pollen deposition either increases or decreases with flower visit. Hence, our general model of pollen deposition is parameterized by five constants, which we denote by $\theta = \{\bar{p}_1, \bar{p}_\infty, \lambda, c, \phi\}$. This model is equivalent to the geometric decay model with a binomial error distribution (eq. 2) when $\bar{p}_1 = \bar{p}_\infty = p$, $\lambda = 0$, $c = 0$, and in the limit as $\phi \rightarrow 0$.

Fitting Pollen Dispersal Data and Model Selection

We used maximum likelihood techniques to estimate the model parameters, θ . Each experimental trial involving dispersal of pollen from a GUS donor by a single pollinator is associated with deposition data, \mathbf{d} . The likelihood of the parameters of the general deposition model, given the data, is

$$L(\theta|\mathbf{d}) = \prod_{v=1}^V \Pr(d_v|\theta), \quad (8a)$$

where $\Pr(d_v|\theta)$ is equation (5) with t replaced by p and R replaced by D . For example, when $c = 0$, the likelihood is

$$\begin{aligned} L(\theta|\mathbf{d}) &= \prod_{v=1}^V \text{betabin}(d_v|D_v, \bar{p}_v, \phi) \\ &= \prod_{v=1}^V \frac{\Gamma(D_v+1)\Gamma(a_v+b_v)\Gamma(d_v+a_v)\Gamma(D_v-d_v+b_v)}{\Gamma(d_v+1)\Gamma(D_v-d_v+1)\Gamma(a_v)\Gamma(b_v)\Gamma(D_v+a_v+b_v)}, \end{aligned} \quad (8b)$$

where $a_v = \bar{p}_v/\phi$, $b_v = (1 - \bar{p}_v)/\phi$, and \bar{p}_v is defined by equation (7). The set of parameter estimates that maximizes equation (8a) is denoted θ^* . We implemented this fitting procedure in Excel using the built-in function GAMMALN() to calculate the log likelihood of each model and the add-in SOLVER to find the maximum likelihood parameter estimates.

To infer patterns in pollen deposition among individual pollinators, we fit the eight models summarized in table 1 to each sequence of pollen dispersal data. These models were derived by setting none of the parameters or some parameters of the general deposition model to 0 (or near 0). Models that assumed stigma contact during all flower visits were derived by setting $c = 0$, whereas models that allowed possible noncontact with stigmas considered $0 \leq c \leq 1$. Models with a constant mean probability of pollen deposition involved $\lambda = 0$, which made \bar{p}_∞ redundant and forced $\bar{p}_v = \bar{p}_1$. Finally, to test whether pollen grains act independently when on a pollinator and/or whether the chance that a donor pollen grain is deposited varied stochastically, we fit both binomial and beta-binomial error distributions by controlling ϕ . If the error variance is most consistent with the beta-binomial distribution, then pollen may have been distributed as clumps and/or pollinator stigma positioning may have varied. In the limit as ϕ approaches 0, the beta-binomial distribution approaches the binomial distribution. Because equation (3) is undefined when $\phi = 0$, models that assumed a binomial distribution were derived by setting $\phi = 10^{-6}$, resulting in $\text{VIF} \approx 1$. Models that assumed a binomial distribution are denoted B, whereas models that assumed a beta-binomial distribution (i.e., ϕ was free to vary) are denoted BB.

Models were selected using Akaike's Information Criterion (AIC), which quantifies model parsimony, reflecting the trade-off between the ability of a model to fit data and its degree of complexity (Burnham and Anderson 2002; Richards 2005; Bolker 2008). For pollen dispersal sequence \mathbf{d} , the AIC value associated with model M is

$$\text{AIC}(\mathbf{M}) = -2 \ln L(\theta_M^*) + 2K, \quad (9)$$

Table 2: Akaike Information Criterion (AIC) model selection results from fitting pollen deposition models to 13 butterfly and 21 bumblebee data sets

Model	<i>Pieris rapae</i>		<i>Bombus melanopygus</i>	
	Times selected	Times best AIC model ($\Delta = 0$)	Times selected	Times best AIC model ($\Delta = 0$)
Binomial:				
B(—)	1	0	0	0
B(<i>c</i>)	1	0	0	0
B(<i>v</i>)	1	1	1	0
B(<i>c</i> + <i>v</i>)	2	0	2	1
Beta-binomial:				
BB(—)	12	10	18	14
BB(<i>c</i>)	2	2	4	1
BB(<i>v</i>)	2	0	7	5
BB(<i>c</i> + <i>v</i>)	0	0	0	0

Note: For each model, we present the number of trials for which the model was selected and the number of selected trials for which the model had the lowest AIC. Models were selected if their Δ value was <6 and no simpler nested model had a lower Δ value.

where θ_M^* is the vector of maximum likelihood parameter estimates associated with model M; $L(\theta_M^*)$ is the maximum likelihood of model M, calculated using equation (8a); and K is the number of estimated parameters. For each model, we calculated Δ , the difference between the model's AIC and the smallest AIC of all eight models. Models with $\Delta < 6$ were initially selected, and to avoid selecting overly complex models, we then excluded models that were more complex versions of the best AIC model (i.e., the model associated with $\Delta = 0$; Richards 2008). Thus, a model was selected only if each of its free parameters increased the maximum log likelihood by at least 1.

Two requirements of model fitting caused us to use only a subset of the dispersal trials for parameter estimation. Estimates will be biased for data from a visitation sequence that did not include enough recipients to deplete the donor pollen carried by the pollinator. To reduce this bias, we chose only dispersal sequences for which at least 20 donor grains were dispersed to recipient stigmas and 95% of the dispersed donor pollen was deposited in the first 75% of recipient flowers. In addition, deposition sequences involving revisits to recipient flowers complicate parameter estimation, because the number of donor pollen grains transferred to a stigma during its first visit and subsequent revisit(s) are unknown, and our models require knowledge of the number of grains remaining to be deposited immediately before each visit (eq. [6]). As a result, we considered dispersal sequences with revisits only if the most likely situation was that all donor pollen was transferred during the first visit to the revisited recipient flower and other possible combinations of pollen transfer to the stigma neither affected which model was selected nor strongly influenced parameter estimates. Pollen sequences for 21 *Bombus melanopygus* (including six trials using re-

cipients with brushed anthers) and 13 *Pieris rapae* trials satisfied all these criteria.

We performed subsequent AIC analyses to test for differences in dispersal characteristics between pollinator species and among individuals of the same species and to assess whether pollen dispersal by bumblebees was affected by the brushing of anthers on recipient plants. To simplify the analysis, we considered only individual trials for which model BB(—) was selected in the initial AIC analysis (i.e., 12 of 13 butterfly runs and 18 of 21 bumblebee runs; see table 2, row 5). Models were constructed by assuming that each pollinator dispersed pollen according to BB(—), and we compared seven models that differed in their assumptions regarding whether \bar{p} and ϕ differed between bees and butterflies, whether anther brushing affected \bar{p} and ϕ , and whether the \bar{p} and ϕ varied among individuals of the same pollinator type or could be better characterized by assuming they were drawn from probability distributions (table 3). For the latter possibility, we assumed that within-group (i.e., within species and/or within anther treatment) variation could be described by assuming that \bar{p} was beta distributed with mean $\mu_{\bar{p}}$ and overdispersion parameter $\delta_{\bar{p}}$ and that ϕ was gamma distributed with mean μ_{ϕ} and variance σ_{ϕ}^2 (see appendix). The likelihood of these four parameters, given deposition data from an individual considered to be part of the group defined by the parameters, is

$$L(\mu_{\bar{p}}, \delta_{\bar{p}}, \mu_{\phi}, \sigma_{\phi}^2 | \mathbf{d}) = \int_{\bar{p}=0}^1 \text{beta}(\bar{p} | \mu_{\bar{p}}, \delta_{\bar{p}}) \int_{\phi=0}^{\infty} \text{gamma}(\phi | \mu_{\phi}, \sigma_{\phi}^2) L(\bar{p}, \phi | \mathbf{d}) d\phi d\bar{p}, \quad (10)$$

Table 3: Akaike Information Criterion (AIC) model selection results when fitting model BB(–) to 30 pollen dispersal trials (12 butterflies and 18 bumblebees)

Model	K	AIC	Δ	Model description
M(–)	2	4,523.7	115.2	All pollinators identical
M(s)	4	4,425.1	16.6	Species differences only
M($s + b$)	6	4,408.5	0	Species differences and brushing effect for bumblebees
M(–) ^a	4	4,453.1	44.6	Common distribution describing parameter variation for butterflies and bumblebees
M(s) ^a	8	4,418.4	9.9	Separate distributions describing parameter variation for butterflies and bumblebees
M($s + b$) ^a	12	4,413.9	5.4	Separate distributions describing parameter variation for butterflies, bumblebees visiting intact recipient plants, and bumblebees visiting recipients with brushed anthers
M(u)	60	4,440.9	32.4	All pollinators have unique dispersal characteristics

Note: Models differed in whether \bar{p} and ϕ were assumed equal for all pollinators (–), were estimated separately for each individual (u), or were determined by species (s), and whether recipient flowers had brushed anthers (b).

^a Indicates models for which pollen dispersal parameters varied among individual pollinators according to specified distributions (\bar{p} , beta distribution parameterized by $\mu_{\bar{p}}$ and $\delta_{\bar{p}}$; ϕ , gamma distribution parameterized by μ_{ϕ} and σ_{ϕ}^2). K is the number of estimated parameters.

where $L(\bar{p}, \phi | \mathbf{d})$ is given by equation (8b). When finding maximum likelihood parameters, we used numerical integration to estimate the integrals in equation (10) (see Bolker 2008).

Simulation of the Effects of Pollen Loss on Parameter Estimates

Our model-fitting procedure does not explicitly assess the incidence of pollen loss, so loss could affect parameter estimates, leading to misinterpretation of results. To address the relations of estimates of \bar{p}_v to actual values of \bar{t}_v and \bar{u}_v and of estimates of ϕ to ϕ_i and ϕ_u , we simulated pollen dispersal with transport loss. Specifically, we assumed that after visiting a donor flower a pollinator carried away $R = 2,000$ donor pollen grains and that pollen on the pollinator before a flower visit was transferred to the flower's stigma with constant mean probability $\bar{t}_v = \bar{t} = 0.04$. Scenarios were investigated that differed in the mean probability that each pollen grain became unavailable (lost) for subsequent deposition, which was assumed to be constant among flowers, including $\bar{u}_v = \bar{u} = 0, 0.04, 0.08, 0.12, 0.16$. Hence, the ratio of pollen lost between flower visits to pollen transferred to stigmas ranged from 0 to 4. Scenarios also differed in the degree of overdispersion, with equal overdispersion parameters for pollen transfer and loss (i.e., $\phi_i = \phi_u = \phi$). Three overdispersion scenarios were investigated ($\phi = 0.01, 0.1, 0.5$), which depicted pollen transfer and loss ranging from being an approximately binomial processes to being highly variable from one flower visit to the next (see fig. 2A). For simplicity, we assumed that pollinators always contacted the stigma during a flower visit (i.e., $c = 0$). The numbers of pollen grains transferred to each stigma and lost between flower visits were randomly drawn according to equation (4). In all cases, pollinators were simulated to visit 250 flowers to ensure that all R grains had been de-

posited. For each distinct scenario, 25 data sets were simulated, and each data set was fit to the deposition model BB(–) using the maximum likelihood techniques described above. Estimates of \bar{p} were then compared with \bar{t} and \bar{u} , and estimates of ϕ were compared with ϕ_i and ϕ_u .

Model of Plant Reproductive Success

We investigated the nature and magnitude of effects of variation in pollen deposition on stigmas and loss between flower visits on average seed set with a numerical model. Suppose a pollinator visits many flowers, each time picking up exactly X pollen grains. Between visits, some pollen grains on the pollinator become unavailable for deposition, according to a beta-binomial distribution with mean loss probability \bar{u} and overdispersion parameter ϕ_u . Similarly, during a visit, the number of grains on the pollinator transferred to the stigma is drawn from a beta-binomial distribution with mean transfer probability \bar{t} and overdispersion parameter ϕ_i . Let $f(i, v)$ be the probability that a pollinator that has visited v flowers carries i pollen grains immediately before visiting the next flower, and let $g(i, v)$ be the probability that the same pollinator carries i pollen grains immediately after visiting flower v . These probability distributions are related according to

$$g(i, v) = \sum_{j=\max(0, i-X)}^{\infty} \text{betabin}(j + X - i | j, \bar{t}, \phi_i) f(j, v - 1), \quad (11)$$

$$f(i, v) = \sum_{j=i}^{\infty} \text{betabin}(j - i | j, \bar{u}, \phi_u) g(j, v). \quad (12)$$

According to equation (11), if the pollinator carried j and i pollen grains immediately before and after the visit, respectively, and X grains were picked up during the visit, then $j + X - i$ grains must have been deposited onto the stigma.

Iteration of these equations for many visits from an initial condition of $f(0, 0) = 1$ and $f(i, 0) = 0$ produces equilibrium probability distributions $g^*(i)$ and $f^*(i)$. When the pollinator then visits an unvisited flower, the probability that the stigma receives i pollen grains is

$$d_1(i) = \sum_{j=i}^{\infty} \text{betabin}(i|j, \bar{t}, \phi_i) f^*(j), \quad (13)$$

where the subscript 1 indicates the flower's first visit. Correspondingly, the distribution of grains deposited on the same stigma after v pollinator visits is

$$d_v(i) = \sum_{j=0}^i d_{v-1}(j) d_1(i-j). \quad (14)$$

For simplicity, we assume that if the stigma receives fewer pollen grains than the number of ovules in the associated ovary, then all grains fertilize ovules; otherwise, the successful grains are chosen randomly. Consequently, if the ovary has O ovules and is visited v times, the expected number of ovules fertilized per flower is

$$\bar{O}_v = \sum_{i=0}^O i d_v(i) + O \sum_{i=O+1}^{\infty} d_v(i), \quad (15)$$

where the first and second terms account for flowers that receive fewer than O pollen grains (fertilizing i ovules) and at least O pollen grains (complete fertilization), respectively. The expected proportion of ovules that are not fertilized is $1 - \bar{O}_v/O$.

Average seed production is pollen limited (i.e., $\bar{O}_v < O$) when some flowers receive fewer pollen grains than their ovule production. Such quantitative pollen limitation could arise from one or two causes. First, if expected pollen receipt is less than the number of ovules, seed production will be limited by insufficient mean pollen import. The resulting expected pollen limitation is $\max\{0, O - \bar{d}_v\}$, where $\bar{d}_v = \sum i d_v(i)$ is the average pollen receipt for flowers visited v times. Second, because of Jensen's inequality, seed production will always be subject to variance limitation of magnitude $\min\{O, \bar{d}_v\} - \bar{O}_v$ when pollen import varies around \bar{d}_v .

Results

Distribution of Pollen Deposition

Dispersal of *Brassica napus* pollen varied extensively between and within pollinator species (fig. 3). Considering just the GUS pollen deposited on the first flower in the sequence of flowers visited by pollinators after they left the transgenic plant, individual bumblebees dispersed al-

most 10-fold more pollen (mean = 126.2 pollen grains, variance = 16,151 grains², lower 95% confidence limit [LCL] = 96.3, upper confidence limit [UCL] = 169.8; fig. 3B) than butterflies (mean = 15.1 grains, variance = 1,052 grains², LCL = 7.7, UCL = 35.8; fig. 3A). If these distributions resulted from a Poisson process, the variance should have equaled the mean, which is clearly not the case. Instead, both samples are consistent with negative binomial distributions (randomization test; butterflies, $P = .45$; bees, $P = .43$), indicating much greater variation than expected for a Poisson process. Overdispersion could have arisen because pollinators picked up differing numbers of pollen grains, grains did not disperse independently of each other, and/or individual butterflies and bees had different dispersal characteristics.

Sequences of dispersal of transgenic pollen by individual pollinators also varied extensively around a generally declining trend (fig. 3C, 3D). Features of variation of particular interest include (1) considerable variation in deposition during visits to initial recipients, when pollinators carry the most donor pollen; (2) sporadic null visits, even relatively early in dispersal sequences; and (3) occasional peaks of deposition late in sequences, when pollinators carried relatively little donor pollen. Some of the differences between pollinator types resulted because bumblebees dispersed approximately seven times more donor pollen (mean \pm SD = 565.3 ± 342.3 grains) than butterflies (80.6 ± 61.0 grains). These sample sequences also reinforce the impression of variation among pollinators of the same species and between species that was evident from the deposition on initial recipients. In particular, butterflies tended to have null visits more often than bees.

Components of Variation in Dispersal of Brassica napus Pollen

For all butterflies and bumblebees, a beta-binomial model was identified as a candidate explanation for their pollen dispersal, and for all but one individual of each species, this distribution provided the best explanation of the observed results (table 2). This result reveals that the probability of deposition varied extensively among flower visits for individuals of both pollinator species (fig. 3E, 3F), in contrast to the expectations for a binomial process. The beta-binomial distribution allows for a high incidence of null visits, especially with large ϕ (e.g., fig. 2A), so it is not surprising that explicit allowance for noncontact between pollinators and stigmas seldom fit the data better than beta-binomial models (table 2).

For both bumblebees and butterflies, model BB(–) was selected most often (table 2), indicating that the mean probability of pollen deposition onto stigmas varied independently of the number of flowers visited since donor

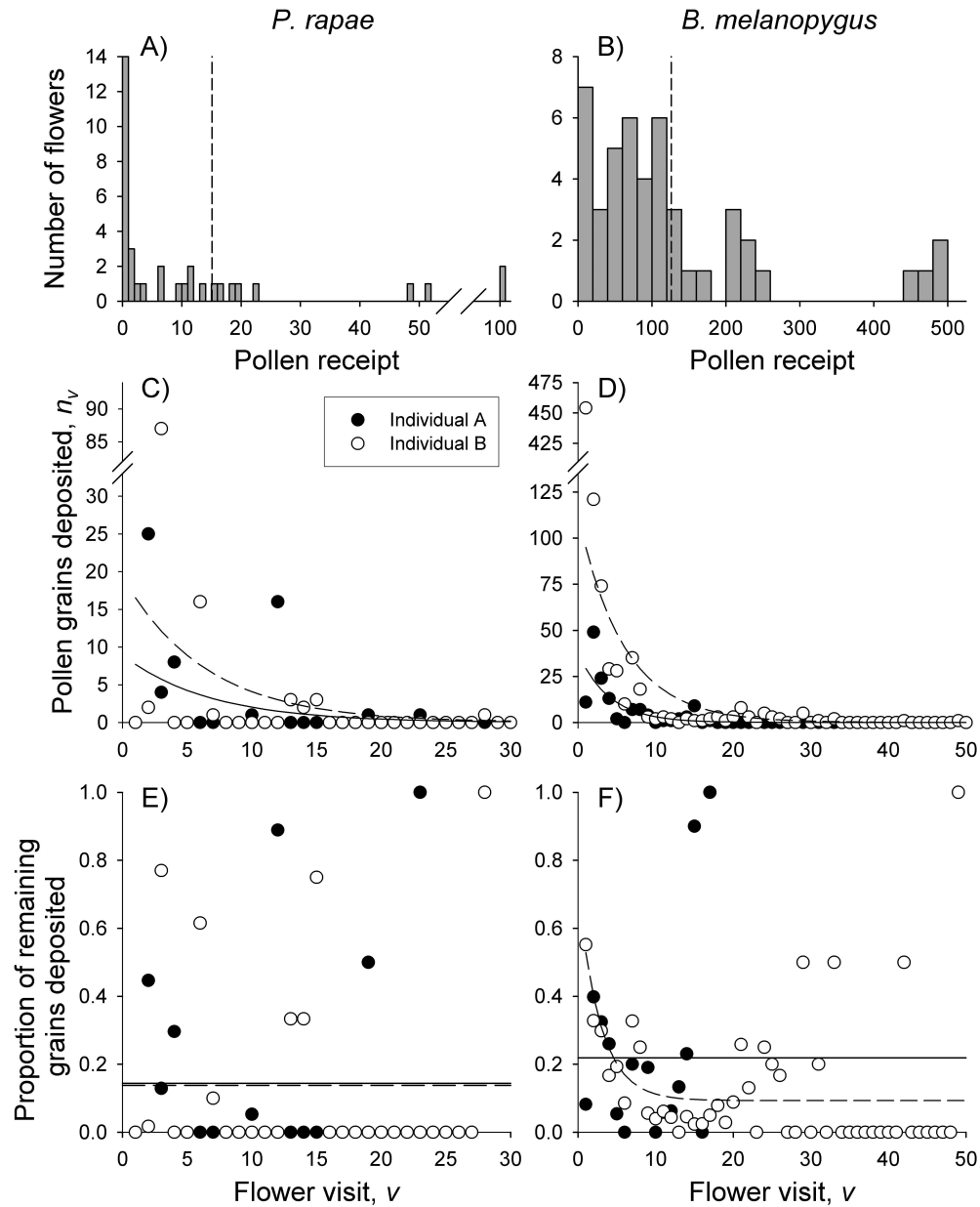


Figure 3: Examples of sources of variation in the dispersal of *Brassica napus* pollen. A and B depict the frequency distributions of the number of pollen grains from transgenic donor plants deposited on stigmas of the first recipient flowers on wild-type plants visited subsequently by 35 *Pieris rapae* and 46 *Bombus melanopygus*, respectively. C and D illustrate entire sequences of dispersal of transgenic pollen to recipient flowers for two butterflies and two bumblebees, respectively. E and F illustrate the corresponding proportion of donor grains deposited per flower. C–F also show the fits associated with the best Akaike Information Criterion model (i.e., $\Delta = 0$). Solid and dashed lines correspond to individuals A and B, respectively.

pollen was picked up. The model that allowed the mean deposition probability to vary systematically with recipient number, $BB(v)$, was selected second most frequently for butterflies (seven of 21 trials) and was the best AIC model for five trials (table 2). In all cases when $BB(v)$ was

selected, the parameter estimates suggest a decline in the mean deposition probability, leveling off between five and 10 flower visits beyond the donor plant (fig. 4). In contrast, although model $BB(v)$ was selected for two of the 15 butterflies, it was never the best AIC model.

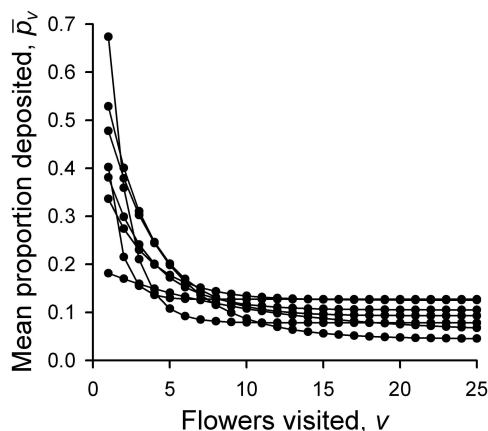


Figure 4: Relations of the estimated mean probability that ultimately dispersed donor pollen grains are deposited on stigmas to a recipient flower's position in the visit sequence for the seven bumblebee runs for which BB(v) was selected as a parsimonious model (see tables 1, 2).

For trials for which model BB($-$) was selected, pollen dispersal differed greatly between bumblebees and butterflies but little among individuals of the same species (see table 3; fig. 5A). Despite numerical differences in the estimates of \bar{p} and ϕ within butterflies and bumblebees with and without brushed anthers (fig. 5A), the interindividual variation was consistent with sampling error (i.e., the data are explained well by very low δ_p and σ_ϕ^2). Both pollinator types had similar \bar{p} , but all butterflies had larger ϕ than the bumblebees (fig. 5A). Consequently, differences between butterflies and bumblebees in dispersal characteristics per pollen grain arise almost entirely from the greater dispersal variance of butterflies. Among trials for bumblebees, those involving recipient flowers with brushed anthers had slightly reduced \bar{p} and significantly smaller ϕ than those with intact recipient flowers (compare confidence intervals in fig. 5A), so again the main difference among classes of trials involved dispersal variance, rather than the average dispersal pattern. Beta distributions corresponding to the maximum-likelihood parameter estimates for these three groups of trials (fig. 5B) illustrate the much greater among-flower variation in deposition probability for butterflies than for either type of trials with bumblebees.

Simulation of the Effects of Pollen Loss on Parameter Estimates

The fits of simulated data generated by model BB($-$) revealed that the mean probability of deposition per flower for pollen that will eventually reach stigmas, \bar{p} , depends on the mean probabilities that a pollen grain on a polli-

nator is deposited on a stigma, \bar{t} , or lost during transit between flowers, \bar{u} (fig. 6A). When we simulated no pollen loss between flower visits ($\bar{u} = 0$) the maximum-likelihood estimate for \bar{p} was an unbiased estimator of \bar{t} . In addition, when overdispersion was limited (small ϕ) the estimated probability of deposition of ultimately dispersed

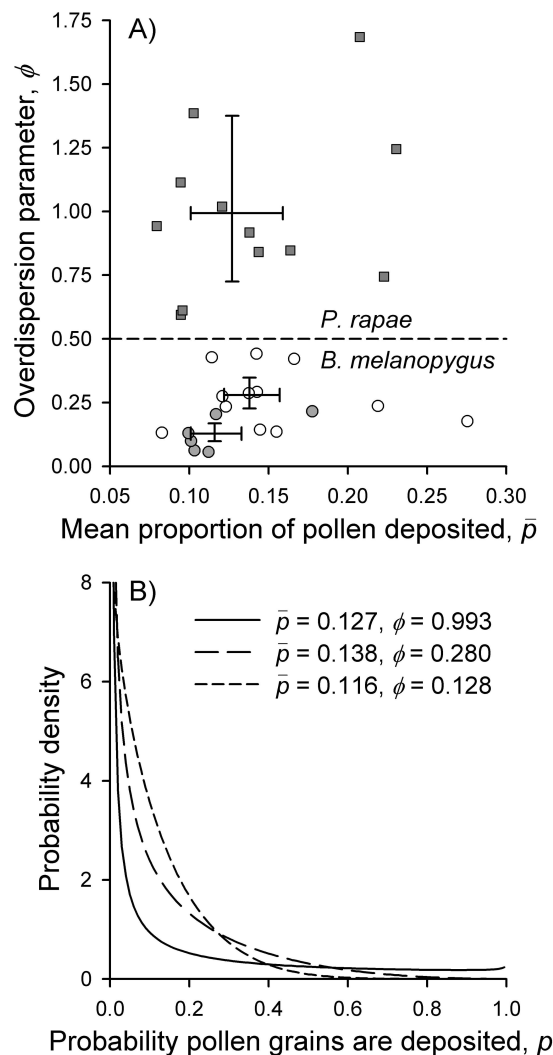


Figure 5: A, Maximum likelihood parameter estimates for individual pollinators where model BB($-$) was selected (18 bumblebees and 12 butterflies; see tables 1, 2). Crosses indicate the estimated 95% confidence interval for the parameter pairs (\bar{p} , ϕ), assuming homogeneity within the three groups of data corresponding to the best Akaike Information Criterion model M($s + b$) that assumes pollen dispersal depends on the pollinator species and whether recipient anthers are brushed (see table 3). B, Beta distributions describing the variation in the probability pollen grains are deposited during each flower visit parameterized by the maximum likelihood estimates for each of the three groups (solid line, butterflies; long-dashed line, bumblebees foraging on unbrushed anthers; short-dashed line, bumblebees foraging on brushed anthers).

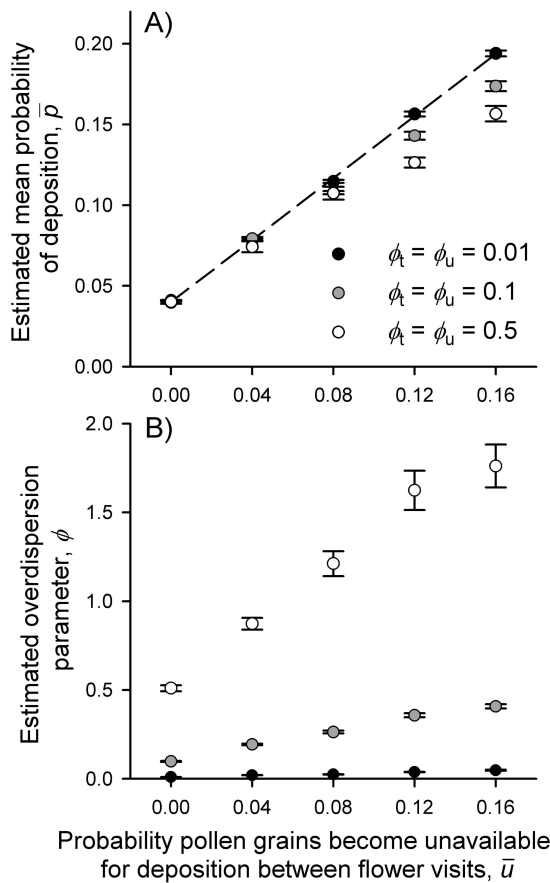


Figure 6: Effects of the average probability of pollen loss during pollinator flights between flowers, \bar{u} , on the mean (\pm SE) maximum likelihood estimate of the proportion of pollen that leaves a pollinator per flower visit, \bar{p} (A), and the maximum likelihood estimate of the overdispersion parameter, ϕ (B), for the beta-binomial model for 25 simulations per parameter set. In all cases, the mean probability that a pollen grain on a pollinator was deposited on a stigma during each flower visit was $\bar{t} = 0.04$. The dashed line in A represents $\bar{p} = \bar{t} + \bar{u}(1 - \bar{t})$, the expectation for a binomial distribution.

pollen, \bar{p} , accurately estimated $\bar{t} + \bar{u}(1 - \bar{t})$. However, as simulated overdispersion and pollen loss increased, \bar{p} increasingly underestimated this relation (fig. 6A). Similarly, in the absence of pollen loss, the maximum likelihood estimate of the overdispersion parameter ϕ was an unbiased estimator of ϕ_t (fig. 6B). The estimated ϕ increased linearly with the probability of pollen loss, \bar{u} , and the rate of increase was proportional to the overdispersion of simulated pollen loss and deposition. In summary, fitted values for both \bar{p} and ϕ characterize combined effects of pollen transfer and pollen loss. Consequently, estimates of these parameters need to be interpreted in terms of the joint effects of both processes rather than with respect to deposition alone (also see Morris et al. 1994; Rademaker

et al. 1997). Developing pollen dispersal experiments that would allow these two loss processes to be estimated independently remains a challenge because it requires estimating the pollen load on the pollinator before and after flower visitation.

Effects of Pollination Variation on Mating Success

Our model of pollen dispersal and mating revealed strong negative effects of variation on mean seed production per flower (fig. 7). For the examples depicted in figure 7, we

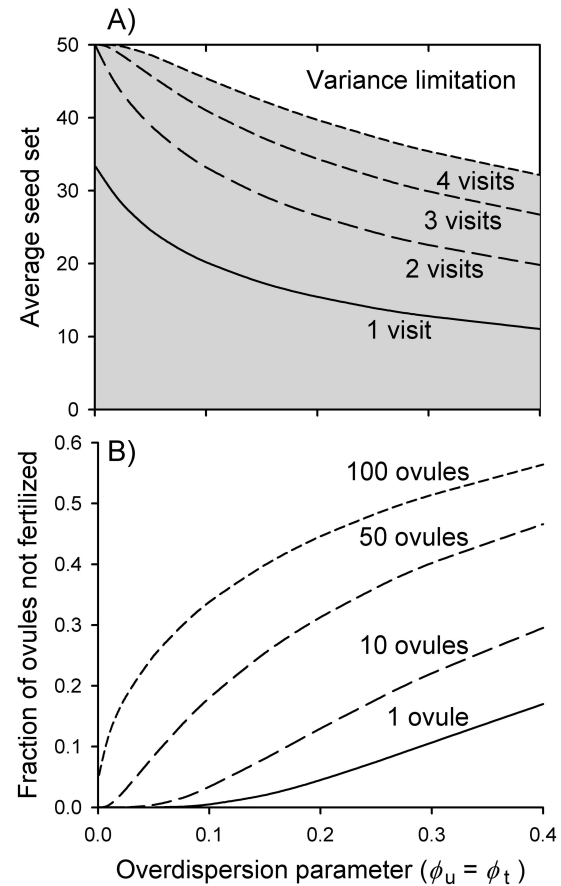


Figure 7: Model results depicting the effects of variation in pollen dispersal on average siring success when pollinators pick up exactly $X = 200$ pollen grains each flower visit and the mean proportions of pollen on pollinators bodies that are lost during flights between flowers and deposited on stigmas are $\bar{u} = 0.16$ and $\bar{t} = 0.04$, respectively. A illustrates the relations of the mean number of seeds sired to the common overdispersion parameters, $\phi_t = \phi_u$, when flowers contain 50 ovules. Results are presented for flowers visited up to four times (lines). The white area labeled variance limitation above the dashed lines depicts the reduction in expected siring success caused by Jensen's inequality. B relates fertilization success with variation in pollen dispersal for flowers with 1, 10, 50, or 100 ovules. All flowers were visited three times.

assumed parameter values describing pollen transfer and loss that were consistent with our bumblebee fits (for details, see legend of fig. 7), which resulted in an average of 34.7 pollen grains being deposited on a stigma per flower visit. Figure 7A depicts cases in which ovaries contain 50 seeds, so flowers experiencing one pollinator visit suffered pollen limitation in part because of insufficient average pollen import, whereas for flowers visited more than once, the average pollen limitation resulted solely from the consequences of Jensen's inequality for variation in pollen import. In these examples, binomial variation in pollen deposition ($\phi \approx 0$) reduced mean seed production very little because most flowers that were visited more than once received sufficient pollen for complete ovule fertilization. However, as the magnitude of between-flower variation in \bar{p} increased so that ϕ was in the range observed for bumblebees ($\phi = 0.1$ to 0.4 and $\bar{p} = 0.1$ to 0.15 ; see fig. 5A), mean seed production fell by up to 80% of capacity. Reductions in seed set were most dramatic when flowers were visited infrequently. However, even when mean pollen deposition per flower exceeded ovule number by 100% ($v = 3$ visits, $\bar{d}_3/O = 2$), seed production was reduced by more than 40%. The much greater overdispersion observed for butterflies (fig. 5A) would further aggravate this depression in average reproductive success. Not surprisingly, variance limitation was less severe for a given ϕ if flowers contained few ovules (fig. 7B) than if they contained many ovules (fig. 7B). However, even with uniovulate flowers, variance limitation caused a 15% average reduction in average seed production for values of ϕ in the range observed for bee pollination, because a large fraction of flowers received no pollen during single pollinator visits (fig. 2A, *dark gray distribution*).

This model also revealed two additional aspects of the role of dispersal variance in mating success. Although the variance in pollen deposition on stigmas, ϕ_p , affected mean fertilization success, variance in transport loss, ϕ_u , did not, since virtually identical results were obtained when we set $\phi_u \approx 0$ rather than $\phi_u = \phi_t$ (not shown). In addition, ovule number and average pollen receipt did not have interacting effects on seed production, so average seed set varied positively with the ratio of expected pollen import to ovule number, O/\bar{d}_v . For example, for a given ϕ_p , when average pollen receipt was twice the number of ovules in the associated ovary, the expected fraction of ovules fertilized was relatively insensitive to ovary size, O .

Discussion

Animal pollination generates extensive variation in pollen dispersal (e.g., fig. 3), which should severely reduce average reproductive performance in plant populations (fig. 7). Given this potential impact, the causes of dispersal vari-

ance become topics of interest because they could depend on ecological circumstances and govern the opportunity for selection on reproductive traits. Interestingly, not all of the three potential sources of dispersal variance that we assessed contributed strongly to variation in the deposition of *Brassica napus* pollen. On the one hand, differences between pollinator types (e.g., nonoverlapping ranges of ϕ for butterflies and bumblebees in fig. 5A) and among visits by individual pollinators (e.g., dominance of beta-binomial models as explanations of the data; table 2) both represent major components of dispersal variance. These differences exist even though the mean probability of ultimately dispersed pollen being deposited on a stigma per flower visit, \bar{p} , was about 0.12 for both pollinator types (fig. 5A). On the other hand, we found no evidence that differences among individuals of the same pollinator type contribute significantly to dispersal variance (table 3). In addition, our results provide little evidence that butterflies and bumblebees visiting *B. napus* failed to contact stigmas with sufficient frequency to be distinguished from the relatively high frequency of null visits expected with stigma contact for values of ϕ in the range observed for bees and, especially, butterflies (fig. 2A, *dark gray distribution*).

Our findings for bumblebees are consistent with those of Cresswell (1999), who measured pollen import during single visits by *Bombus lapidarius* workers to *B. napus* flowers with intact or brushed anthers. Cresswell did not track marked pollen from a specific donor, so his data for flowers with brushed anthers (which could receive only outcross pollen) are most comparable to our results for the first flower that bees visited after leaving donor plants. Cresswell's mean of 141 pollen grains for flowers with brushed anthers falls well within the confidence interval for our mean of 126 grains, indicating equivalent pollen dispersal. In addition, the variance : mean ratio for Cresswell's data was 36, indicating much more variation than expected from a Poisson distribution, and it is consistent with the negative binomial distribution that characterized our bumblebee data.

Our results are largely inconsistent with a strong systematic decline in the mean probability of deposition of donor pollen as pollinators move away from donor plants. This conclusion differs from that of some previous studies (e.g., Morris et al. 1994; Harder and Barrett 1996), which could occur because dispersal of *B. napus* pollen differs from dispersal for other species and/or the application of different statistical methods led to contrasting conclusions. These alternatives cannot be distinguished without analyzing data for various species with our methods; however, several aspects of the effect of dispersal variance on model fitting raise the possibility that this contrast has a statistical basis. Therefore, before considering the biological implications of our results, we address the importance of ac-

counting correctly for variance during statistical analysis of pollen dispersal.

Considerations for Fitting Pollen Dispersal Models

Fitting nonlinear, process-based models requires both the formulation of models that characterize relevant biological mechanisms and the inclusion of the appropriate sampling distribution to represent the mean, variance, and higher moments adequately as well as their interdependence, if any (Bolker 2008). Mechanistic models of expected pollen dispersal from individual donor plants have been the subject of considerable discussion (e.g., Bateman 1947; Lertzman and Gass 1983; Morris et al. 1995; Rademaker et al. 1997; Harder and Wilson 1998), but the nature of variation around the expectation has not been considered. Furthermore, although the proposed mechanistic models are conceptually useful, some include parameters that cannot be estimated with confidence with only counts of pollen loads on stigmas (e.g., Harder and Wilson's [1998] models of the effects of pollinator grooming and layering on pollinators' bodies). This problem required us to develop the set of more phenomenological models considered in this article (see also Morris et al. 1994) and to use simulation to assess the likely effects on model fitting of processes that could not be measured directly (e.g., transport loss).

The most common method of fitting dispersal data uses standard linear regression techniques after log transformation of the stigmatic pollen counts to fit Bateman's model (eq. [1]) or its exponential equivalent (e.g., Waser and Price 1984; Thomson 1986; Rademaker et al. 1997). This approach assumes a lognormal distribution of deposition around the mean for each recipient position. In contrast, the example distributions in fig. 3A and B are inconsistent with a lognormal distribution, because receipt of no pollen is the most common outcome and the variance is too large, so that log transformation (or any monotonic transformation) would not produce the normal error distribution required by standard regression analysis. Alternatively, data from separate trials could be averaged for each recipient position, which, according to the central limit theorem, should result in a normal distribution of means for a reasonable number of trials (e.g., Harder and Barrett 1996); however, averaging can lead to the impression that the deposition fraction declines systematically with recipient position, even though it is constant for individual trials (Harder and Wilson 1998). Furthermore, the variance arises from the processes responsible for the dispersal pattern, so that analysis of averages ignores aspects of the data that might be informative. Therefore, analysis of pollen dispersal should use individual rather than averaged observations and incorporate the distribution that reflects the nature of the observations, which

will usually require the application of techniques other than regression based on the normal distribution.

Pollen counts on stigmas are nonnegative integers, so only discrete distributions are formally appropriate. We modeled pollen deposition as though the number of (ultimately dispersed) pollen grains on the pollinator awaiting deposition was known (known total approach), in which case the binomial or beta-binomial distributions are reasonable options, depending on the observed variance. Alternatively, deposition could be modeled as though the number of pollen grains awaiting deposition is unknown (unknown total approach), in which case the Poisson or negative binomial distributions are appropriate (e.g., Morris et al. 1994), again depending on the variance (see "Distribution of Pollen Deposition"). We compared known total and unknown total approaches (results not shown) and found the former to be much superior, on the basis of AIC, because it uses available information about the number of pollen grains that will eventually be dispersed before each visit to a recipient flower. Regardless of which approach is used, choosing a sampling distribution that correctly characterizes the magnitude of variation is important, because an incorrect choice can lead to selection of an incorrect model for the average dispersal pattern. For example, if we ignore the overdispersion of the deposition data compared with a binomial distribution and perform AIC analysis considering only binomial models $B(-)$, $B(c)$, $B(v)$ and $B(c + v)$, then the most complex model, $B(c + v)$, is selected exclusively for eight of the 13 butterfly trials, and model $B(-)$ is not selected for any trial. This mistake arises when overdispersion is ignored because overly complex models allow more flexibility in the mean to accommodate small or large observations (including frequent null visits) that are too extreme to be consistent with typical variation for the assumed variance model (for general discussion of this problem, see Richards 2008). Similar biased selection of overly complex models may also have contributed to Morris et al.'s (1994) report of declining deposition fractions, if negative binomial distributions (e.g., fig. 3A, 3B) were more appropriate than the Poisson distributions that they considered.

Consequences of Variation in Pollen Dispersal

Nonlinear conversion of pollen receipt into seed production and variation in pollen dispersal may combine to limit seed production. This limit may occur even when flowers receive sufficient pollen to fertilize all ovules if it had been distributed more evenly among flowers (fig. 7). In our mating model, all pollen grains on stigmas fertilized ovules when total pollen receipt (i) was less than or equal to ovule availability (O), so that the probability that at least one ovule in an ovary is not fertilized equals the cumu-

lative probability that $i < O$ grains were deposited, $F = \sum_{i=0}^{O-1} d_v(i)$. When the expected number of fertilizations was greater than the number of ovules ($\bar{d}_v > O$) and pollen deposition exhibited binomial variation, this cumulative probability was always effectively 0, because average pollen import greatly exceeded ovule number and realized import varied relatively little (see fig. 2B), so that variance limitation did not occur (left edge of fig. 7A when $v > 1$). In contrast, the greater variance of beta-binomial distributions increased the chance that $i < O$ for the same average pollen import, increasing the probability of variance limitation (remainder of fig. 7A). Ovule limitation varies positively with ovule number; however, variance limitation is evident even for uniovulate flowers for ϕ less than that observed for pollination by bumblebees and butterflies (fig. 7B). Thus, variance limitation probably occurs commonly and so acts as a pervasive influence on reproductive performance in plant populations and floral evolution.

The general consequences of variance limitation differ between female and male function. From the female perspective, variance limitation would decline or be eliminated if individual flowers received multiple visits (fig. 7A). In contrast, multiple visits cannot mitigate the consequences of variance limitation for male success, because siring opportunities lost when a donor contributes pollen to stigmas that receive more pollen than needed to fertilize all ovules cannot be shifted to stigmas that receive too few grains for complete fertilization. Thus, for ecological performance, which depends primarily on seed production, variance limitation will be most important when flowers typically receive few pollinator visits and so could aggravate pollen limitation. From an evolutionary perspective, effects of variance limitation on female function should arise in populations with limited pollinator service, whereas its evolutionary effects on male function should occur more generally. Such asymmetric relations between the sex roles are the essential aspect of Bateman's (1948) principle that drives sexual selection.

Variance limitation could select for three types of characteristics: floral traits that increase pollinator visitation, reduce dispersal variance, or reduce the nonlinearities in postpollination processes that cause Jensen's inequality. Traits that could reduce dispersal variance include pollinator specialization and/or floral mechanisms that enhance the precision of interactions between pollinators and floral sex organs. Dispersal variance caused by a diverse fauna of pollinators that differ in the variance they create should promote specialization for pollination by the low-variance pollinators. For example, our empirical results indicate that butterflies are high-variance pollinators of *B. napus* (fig. 5A), so that in *B. napus* populations served by both butterflies and bumblebees, selection should favor specialization on bee pollination. In contrast to Stebbins'

(1970) hypothesis that selection favors specialization on the most effective pollinator (i.e., that providing the highest average pollination), specialization could evolve in response to differences in the variance in dispersal among pollinators, even if the low-variance type provided somewhat lower average pollen dispersal. The variance-reducing advantages of increased pollinator attraction arise largely from the considerations outlined in the preceding paragraph, which emphasized that such advantages arise primarily through female function when seed production is susceptible to variance limitation. In contrast, selection favoring precise pollinator-flower interactions could act through both sex roles. Such selection should lead to the origin and refinement of traits such as zygomorphy, concealed nectar, buzz pollination, heterostyly, and enantio-styly. Furthermore, selection for precise pollinator-flower interactions may commonly accompany pollinator specialization (see Muchhala 2007), synergistically reducing two components of dispersal variance. Strangely, although selection for precision has long been recognized as a recurring theme in angiosperm evolution (see Neal et al. 1998) and precision specifically denotes "low variance," variation in pollen export and import has seldom been a topic of explicit interest (although see Waser and Price 1984).

The second class of adaptations that reduce Jensen's inequality affect the postpollination processes that cause nonlinear conversion of pollen dispersal into fitness. In contrast to variance reduction adaptations, most of the causes of diminishing returns after pollination involve female traits, including limited stigma capacity for pollen receipt, limited style capacity for transmission of pollen tubes, and limited ovule number. For example, Burd (1995) demonstrated theoretically that stochastic pollination may affect the evolution of production of "excess" ovules as a bet-hedging strategy to take advantage of occasional receipt of many pollen grains. In contrast, we found that greater ovule numbers generally aggravated variance limitation of seed production (compare curves in fig. 7B for a given ϕ), although our model did not incorporate the allocation trade-offs between ovule and flower size and number that Burd considered. Regardless, this example emphasizes that selection on sex allocation may interact with selection on floral traits that influence dispersal variance, again revealing the importance of considering variance during pollen dispersal.

From both female and male perspectives, pollen grains on stigmas do not represent reproductive success; instead, they are means to that end. Because pollination is a high-variance process (e.g., figs. 3A, 3B, 5A) and postpollination processes typically impose nonlinear conversion of pollen grains on stigmas into genetic contributions to seeds (e.g., fig. 1), Jensen's inequality must commonly reduce average

reproductive success below that expected from average pollen dispersal. Given the likely ecological and evolutionary implications of this effect outlined above, dispersal variance cannot be considered simply noise that is an inevitable consequence of relying on pollen vectors that are disinterested in promoting plant reproduction. Instead, dispersal variance is a characteristic of pollination with unique consequences and so deserves to be recognized as a pervasive and significant factor in angiosperm reproduction.

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APPENDIX

Complete Gamma Function

The complete gamma function for real x is defined by the integral

$$\Gamma(x) = \int_{t=0}^{\infty} t^{x-1} e^{-t} dt.$$

If n is an integer, $\Gamma(n) = (n-1)!$.

Gamma Distribution

The probability density function for variable X when distributed according to the gamma distribution having mean μ and variance σ^2 is

$$\text{gamma}(X = x | \mu, \sigma^2) = \frac{x^{k-1} \exp(-x/\theta)}{\Gamma(k)\theta^k},$$

where Γ is the complete gamma function (see above), $k = \mu^2/\sigma^2$, and $\theta = \sigma^2/\mu$.

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